CLAIMS

1. An Insulator construct for controlling leaky expression of a lethal gene from enhancing functions of a strong constitutive promoter present in the said Insulator construct following integration into the genome of a plant, said Insulator construct comprising :

   i) first transcription unit comprising a lethal gene under transcriptional control of a tissue specific promoter for targeted expression in specific tissue(s) and fused to a suitable transcription termination signal, including a polyadenylation signal,

   ii) second transcription unit comprising a selectable marker DNA under transcriptional control of a strong constitutive promoter with a leader sequence and fused to a suitable transcription termination signal, including a polyadenylation signal,

   iii) an Insulator sequence placed between the first and second transcription units so as to distance the first transcription unit from enhancing functions of the constitutively expressing promoter in the second transcription unit.

2. A construct as claimed in claim 1 wherein the lethal gene of the first transcription unit represents any coding sequence, which, upon expression in a plant cell, significantly disrupts the normal metabolism, function or development of the cell thereby leading to death of the cell.

3. A construct as claimed in claim 1 wherein the lethal gene is selected from the group comprising *barnase*, *RnaseT1*, *binase*, *rolB*, *rolC* and Diphtheria toxin A chain-coding gene.

4. A construct as claimed in claim 1 wherein the preferred lethal gene is *barnase*.

5. A construct as claimed in claim 1 wherein the tissue specific promoter of first transcription unit is selected from the group comprising TA29, A9, A3, tap1, bcp1 and napin.

6. A construct as claimed in claim 1 wherein the preferred tissue specific promoter is TA29.

7. A construct as claimed in claim 1 wherein the marker gene of the second transcription unit is selected from the group comprising *bar*, *ALS*, *tfdA*, *nptII*, *hpt* and *aadA*.

8. The construct as claimed in claim 1 wherein the preferred marker gene is *bar*.

9. The construct as claimed in claim 1 wherein the strong constitutive promoter for expression of the *bar* gene is CaMV35S promoter.

10. The construct as claimed in claim 1 wherein the Insulator sequence comprises a sequence derived from genomic DNA of a plant.

11. The construct as claimed in claim 1 wherein the Insulator sequence has a length of at least 2kb.

12. The construct as claimed in claim 1 wherein the preferred length of the Insulator sequence is about 5kb.

13. A male sterile transgenic plant and parts or seeds thereof which contain in their nuclear genome the construct of claim 1.

14. The plant as claimed in claim 13 which is selected from the group of dicotyledonous or monocotyledonous plants.

15. The preferred plant as claimed in claim 13 is a dicotyledonous plant *Brassica juncea*.

16. A method to obtain male-sterile plants, said method comprising the steps of :

i) transforming the nuclear genome of plant cells with a foreign DNA comprising:

a) a first transcription unit comprising a lethal gene under transcriptional control of a tissue specific promoter end for targeted expression in specific

tissue(s) and fused to a suitable transcription termination signal, including a polyadenylation signal,

b) a second transcriptional unit comprising a selectable marker DNA under transcriptional control of a strong constitutive promoter with a leader sequence and fused to a suitable transcription termination signal, including a polyadenylation signal,

c) an Insulator DNA sequence derived from plant genomic sequences, placed between the first and second transcription units, so as to distance the first transcription unit from enhancing functions of the constitutive promoter in the second transcription unit.

ii) regenerating plants from said transformed plant cells,

iii) identification of male sterile transgenic plants by morphological observations and by their failure to set seed on selfing,

iv) obtaining, at a high frequency, male sterile plants with normal vegetative morphology and normal female fertility,

v) identifying single copy male sterile lines by Southern hybridization,

vi) backcrossing male sterile plants with untransformed parent to obtain T1 seeds,

vii) obtaining male sterile plants with normal T1 seed germination frequencies

viii) obtaining normal segregation ratio of marker gene among T1 progeny of single copy male sterile plants identified,

ix) obtaining stable transfer of male sterile phenotype among all T1 plants exhibiting marker resistance.

17. A method as claimed in claim 16 wherein the preferred lethal gene is *barnase*.

18. A method as claimed in claim 16 wherein the preferred tissue specific promoter is TA29.

19. A method as claimed in claim 16 wherein the preferred marker gene is *bar*.

20. A method as claimed in claim 16 wherein the preferred constitutive promoter is CaMV35S promoter.

21. A method as claimed in claim 16 wherein the preferred length of the Insulator sequence is about 5kb.

22. A method as claimed in claim 16 wherein the plant used for transformation is a dicotyledonous plant *Brassica juncea*.

23. A method as claimed in claim 16 wherein male sterile lines in *Brassica juncea* are generated preferably by *Agrobacterium*-mediated transformation using disarmed Ti plasmid.

24. A method as claimed in claim 16 wherein the male sterile transgenic plants are backcrossed to the untransformed parent and tested for female fertility as evidenced by their ability to set seed in crosses.

25. A method as claimed in claim 16 wherein the male sterile plants are analyzed by Southern hybridization to identify transgenic plants containing a single copy of the T-DNA insert.

26. A method as claimed in claim 16 wherein seeds obtained from backcrossing the above male sterile plants are tested for their viability as evidenced by their ability to germinate on non-selective media.

27. A method as claimed in claim 16 wherein germinated seedlings obtained from backcrossed seeds were tested for segregation of the marker gene by transferring them on selective media.

28. A method as claimed in claim 16 wherein the T1 plants obtained from selected backcrossed progeny were transferred to field conditions and tested for stable inheritance of the male sterile phenotype.